Spatial and Diel Variability in Photosynthetic and Photoprotective Pigments in Shallow Benthic Communities

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LONG-TERM GOALS

Our overall goal is to understand how photosynthetic and photoprotective pigments in benthic plants (primarily benthic microalgae) affect the optical properties (primarily spectral reflectance and fluorescence) of shallow benthic environments. The information gained will be used for the development and testing of rapid scanning optical techniques for detecting and assessing changes and specific disturbances in benthic communities.

OBJECTIVES

Our main objective is to determine the spatial and temporal (particularly diel) variation in a variety of photosynthetic and photoprotective pigments and examine how these pigments affect the spectral reflectance and fluorescence at the sediment surface. An understanding of these relationships is needed in order to refine algorithms used for processing data collected with various multispectral and hyperspectral imaging instruments used for identification and characterization of both living and man-made objects in shallow benthic environments.

APPROACH

Sediment cores with overlying water were collected at various sites around Lee Stocking Island, Bahamas and transported back to the field lab within hours. Study sites included the following: channel marker area with strong currents and no seagrass (CMs), near channel marker in sparse seagrass bed (CMt), near channel marker in dense seagrass bed (CMd), Rainbow Gardens in area without seagrass (RGs), Rainbow Gardens in area with dense seagrass bed (RGg), grapestone with thin biofilm near Norman's pond (WGP) (NWGP), grapestone with dense yellow biofilm near Norman's pond (YGP) (NYGP), top of burrowing shrimp mound at Twin Beaches (TBm) and around mound (TBf), area with green biofilm near Twin Beaches (TBgf), area with dense biofilm near North Perry reef (NP), Coconut Beach near shore with dense biofilm (CMnf) and progressively farther from shore

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14. ABSTRACT
Our overall goal is to understand how photosynthetic and photoprotective pigments in benthic plants (primarily benthic microalgae) affect the optical properties (primarily spectral reflectance and fluorescence) of shallow benthic environments. The information gained will be used for the development and testing of rapid scanning optical techniques for detecting and assessing changes and specific disturbances in benthic communities.

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(CBmf) (CBf), Long Cay in sparse seagrass bed (LET), and Rainbow South outside seagrass bed (RStr) and within seagrass bed (RGg).

Spectral fluorescence and reflectance was measured back in the field lab at the surface of the cores while inside a light-tight container. Spectral fluorescence was measured with a SPEX Fluorolog-3 spectrofluorometer with a fiber optic probe held 10 mm from the sediment surface with a X-Y-Z positioner, and with an Ocean Optics S2000-FL fluorometer with a fiber optic probe held 5 mm from the sediment surface. Spectral reflectance was measured with an Ocean Optics S2000 UV-VIS spectrometer with a fiber optic probe held perpendicular and 25 mm from the sediment surface. The surfaces of sediments cores were also examined and photographed with an Olympus SZX-12 stereo microscope to document grain characteristics, microalgal communities, and extracellular polymeric substances at the sediment surface.

Reflectance (R) was calculated relative to a calibrated WS-1 Spectralon (LabSphere) diffuse reflectance standard (99% reflective). Raw reflectance data were smoothed using a cubic spline, pre-averaged with 60 nodes and a number of points equal to the original data set (2048 points). Subsequently, 1^{st} and 2^{nd} derivatives of reflectance spectra were calculated using 7 nm intervals. Second derivative spectra were used, along with published *in vivo* absorption peaks for individual pigments, to identify wavelengths in the reflectance spectra that were most affected by specific pigments. The 2^{nd} derivative (slope) for a 7 nm waveband which corresponded to absorption by a specific pigment was divided by reflectance at the wavelength most affected by that pigment (as identified by 2^{nd} derivative analysis). This normalization of 2^{nd} derivatives to reflectance was necessary to correct for decreases in reflectance across the spectrum caused by varying amounts of extracellular polysaccharides (EPS) produced by the microalgae at different sampling sites.

For pigment analyses, the top 5 mm of the cores were removed and frozen in liquid nitrogen. For fluorometeric chlorophyll analysis, the sediments were extracted repeatedly with 90% acetone until little pigment was observed in the last extract. Fluorescence of the samples was measured before and after acidification with a Turner 10-000R fluorometer. For phycoerythrin and phycocyanin analyses, the sediments were extracted repeatedly with a phosphate buffer. An ultrasonicator was used to extract pigments. Concentrations were determined with a SPEX Fluorolog-3 spectrofluorometer calibrated with pure pigments obtained from Sigma Chemical Co. Chlorophylls a, b, and c were also determined with the SPEX spectrofluorometer. For high performance liquid chromatographic (HPLC) chlorophyll and carotenoid analyses, the cores were extracted in 100% acetone and analyzed using a Hewlett Packard High Performance Liquid Chromatograph attached to a diode array detector. Photosynthetic and photoprotective pigments were identified and quantified by comparing with standards purchased from VKI.

For examination of diel variation, cores were incubated in outdoor water tables with flowing seawater. At various times of the day and night, these cores were placed in a light tight box and their spectral fluorescence and spectral reflectance measured using a fiber optic probe as above. For *in situ* fluorescence measurements, a WET Labs ECO-DFLB underwater fluorometer was placed in light tight cradles implanted in the sediments at various times of the day. The cradles were designed so that the sediment surface received natural sunlight except for the 2 minutes when the fluorometer was placed in the cradle. In addition to fluorescence of algae at the sediment surface, *in vivo* fluorescence was also measured in the water column of large concrete 1 meter deep aquaculture tanks on a diel basis for comparison.

WORK COMPLETED

We have completed our field sampling and all of our sample processing for all years. Analysis and comparison of all the data is ongoing.

RESULTS

Considerable spatial variation exists at various locations. At Lee Stocking Island in the Bahamas, ooids have chlorophyll concentrations around 10-15 mg m^{-2}. Freshly excavated shrimp mounds also have low chlorophyll concentrations in this range. Offshore, calcareous sands near North Perry reef ranged from 11 to 25 mg m^{-2} but offshore muds in the area had chlorophyll concentrations around 33-41 mg m^{-2}. A variety of inshore sediments around Lee Stocking Island had chlorophyll concentrations ranging from 14 to 68 mg m^{-2} The areas around Lee Stocking Island with the largest benthic microalgal biomass were grapestone sediments, with chlorophyll concentrations of 73-104 mg m^{-2}, and Norman's Pond with concentrations of 57-177 mg m^{-2}. Phycoerythrin:chlorophyll and phycocyanin:chlorophyll ratios exhibit a wide range, indicating the proportion of prokaryotic cyanobacteria to eukaryotic algae varies considerably in the sediment surface of these habitats.

Among 66 core samples taken in April, 2001, there was approximately a 10-fold range in concentrations of chlorophyll *a* among the different habitats, as has been observed in previous years. Chlorophyll *c*, on the other hand, exhibited a 300-fold range, indicating considerable variation in species composition. While phycoerythrin concentrations varied around 20-fold, phycocyanin varied approximately 70-fold. The highest levels of chlorophylls *a* and *c*, and phycocyanin were found in grapestone sediments and the lowest levels of chlorophylls *a* and *c* were found in ooid sands. Considerable variation in species composition is reflected in the wide range of pigment ratios: 46 for chl *c*/chl *a*; 94 for phycoerythrin/chl *a*; and 27 for phycocyanin/chl *a*.

Reflectance across the spectrum (400-710 nm) (Fig. 1) was highest at the TBm, CMs, and CMt sites where biomass (chlorophyll *a* plus chlorophyllide *a*) was low (<2 μg g^{-1}), and lowest at the NP site, where biomass was almost an order of magnitude higher (17.7 μg g^{-1}). However, at the WGP site, biomass (2.0 μg g^{-1}) was similar to the first 3 sites, but the overall reflectance was much lower. These observations, combined with results of studies conducted in collaboration with Alan Decho, Pam Reid and Eric Louchard, indicate that while benthic microalgal pigments do affect the overall magnitude of spectral reflectance, other factors such as extracellular polysaccharide secretions may be equally or more important in some areas.

Microalgal pigments affect the shape, as well as the magnitude, of reflectance spectra. Second derivative analyses of reflectance spectra can be used to identify narrow wavebands that are most affected by specific groups of microalgal pigments. Ten narrow wavebands (ca. 7 nm) within the reflectance spectra were identified; 9 of which can be related to specific groups of pigments. Results of linear regression analyses of the ratio of the 2^{nd} derivatives to reflectance plotted against the concentrations of corresponding pigments indicate that measurements of hyperspectral reflectance of sediments may be useful for distinguishing major benthic habitat types and for monitoring large changes over time in benthic microalgal communities (Fig. 2).

As observed and reported before, we observe in incubated cores a large variation in chlorophyll fluorescence over the diel cycle. No significant diel variation is observed in spectral reflectance. This suggests that chlorophyll was constant over the diel cycle and all the fluorescence variation is due to

photoadaptation. Specifically, nonphotochemical quenching can probably explain the large drop in chlorophyll fluorescence during the day with no significant change in chlorophyll concentration.

IMPACT/APPLICATIONS

These data indicate that it should be possible to characterize benthic microalgal communities using remote sensing.

TRANSITIONS

The information gained from these studies is being used by other CoBOP investigators to create spectral libraries of different habitat types and for modeling efforts aimed toward interpretation of PHILLS images made during the same study period.

RELATED PROJECTS

In a project funded by NOAA, we are examining the spatial and temporal distribution of benthic microalgae in Florida Bay and their relationship to nutrient and phytoplankton distributions.

PUBLICATIONS

Brand, L.E. 2000. *Spatial and temporal variation in the optical properties of benthic microalgae*. Ocean Optics XV (abstract).

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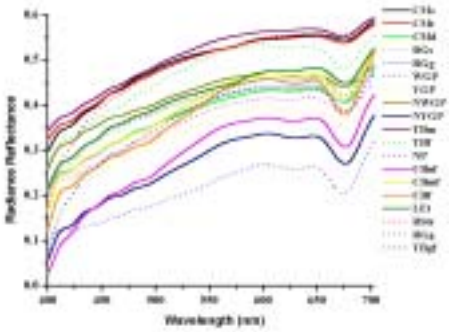

Figure 1. Hyperspectral reflectance (400-710 nm) at 18 sites near LSI. Abbreviation for sites same as in text. [Among sites, ranges in reflectance at specific wavelengths were as follows: 444 nm (0.14-0.42), 492 nm (0.17-0.47), 540 nm (0.21-0.53), 767 nm (0.20-0.55).]

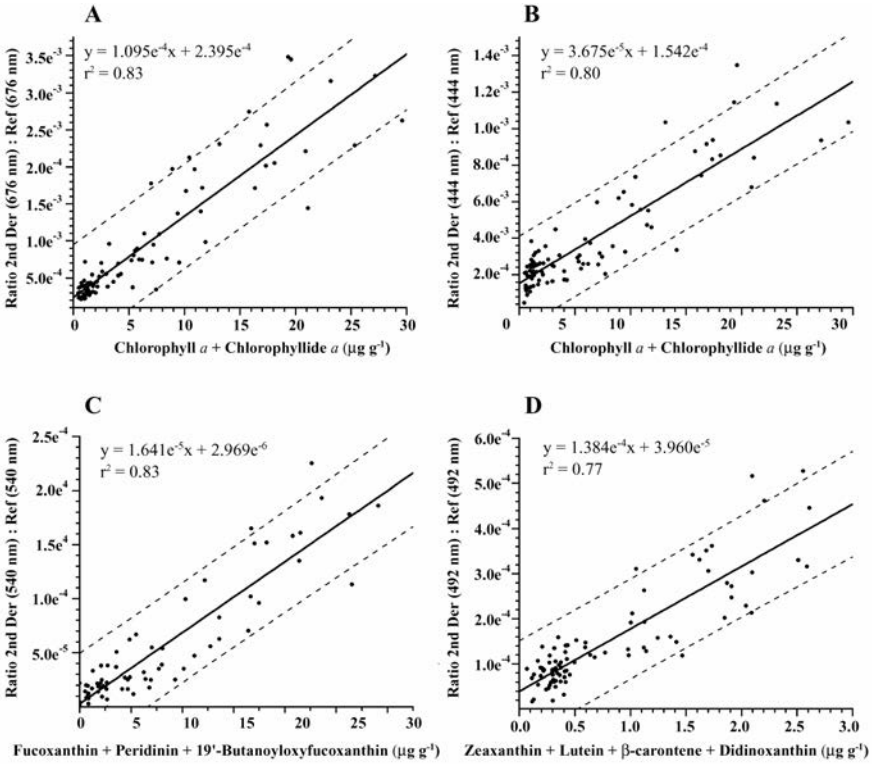

Figure 2. Linear regressions of the ratio of 2nd derivatives to reflectance versus pigment concentrations. Dashed lines, 95% prediction interval. Panels as follows: A, 2nd derivative:reflectance at 676 nm vs. chlorophyll a plus chlorophyllide a (r^2 0.83); B, 2nd derivative:reflectance at 444 nm vs. chlorophyll a plus chlorophyllide a (r^2 0.80); C, 2nd derivative:reflectance at 540 nm vs. the sum of fucoxanthin, peridinin, and 19'butanoyloxyfucoxanthin (r^2 0.83); D, 2nd derivative:reflectance at 492 nm vs. the sum of zeaxanthin, lutein, β-carotene and diadinoxanthin (r^2 0.77). [The sum of chlorophyll a and chlorophyllide a could be estimated from regressions at either 444 or 676 nm within about 1 order of magnitude at concentrations less than 10 µg g^{-1}, and within a factor of 2 between 10-30 µg g^{-1}. The sum of fucoxanthin, peridinin, and 19'-butanoyloxyfucoxanthin could be estimated with similar accuracy at 540 nm. The sum of zeaxanthin, lutein, β-carotene, and diadinoxanthin could be estimated within 1.5-2 orders of magnitude from reflectance at 492 nm at the low concentrations (less than 3 µg g^{-1}) present in our samples.]